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Correlating MALDI and MRI Biomarkers of Breast Cancer

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14. ABSTRACT The processes of tumor growth and treatment response are associated with the up-regulation of numerous proteins, yet current clinical imaging methods of cancer characterization monitor only gross morphology. This study combines specialized in vivo magnetic resonance imaging (MRI) with matrix-assisted laser desorption ionization (MALDI) analysis of healthy and tumorous ex vivo specimens in order to examine the proteomic influences on contrast in MRI. During the current research period, protocols were developed to image and correlate data from breast cancer metastases to bone. MRI data acquisition was expanded from that of the previous research period to include gadolinium contrast-enhanced, diffusion-weighted, and relaxometric data in an intra-tibial mouse model of metastatic breast cancer. Multi-parametric MRI data were collected for eight mice at each of three time points. Acquisition of MALDI data for each mouse is currently underway. Coregistration of proteomic and MRI hind limb data will incorporate both rigid and non-rigid methods in order to fuse the two datasets based on fiducial markers in the absence of a non-deformable stereotactic frame. Data analysis will focus on identification of specific (groups of) proteins that most strongly correlate with variations in multi-parametric MRI data. This work represents a basic yet vital step towards the long-term objective of facilitating clinical assessment of tumor status via non-invasive imaging techniques.				
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## INTRODUCTION

THIS REPORT SUMMARIZES THE WORK AND RESULTS OF THE SECOND OF THREE YEARS OF TRAINING FUNDED BY THIS GRANT. THIS GRANT SUPPORTS THE PI'S PREPARATION FOR A CAREER IN CANCER RESEARCH BY PROVIDING FOR TRAINING IN IMAGE PROCESSING, ANALYTICAL METHODS, AND THE-ART LABORATORY TECHNIQUES USED IN CANCER RESEARCH. THE PURPOSE OF THE FUNDED TRAINING IS TO ASSESS THE CORRELATION BETWEEN PHYSIOLOGICAL PARAMETERS REPORTED BY MAGNETIC RESONANCE IMAGING AND TUMOR PROTEIN DISTRIBUTION DETERMINED FROM MATRIX-ASSISTED LASER DESORPTION/IONIZATION (MALDI) MASS SPECTROMETRY MEASUREMENTS. THE OVERARCHING GOAL OF THIS TRAINING IS TO ELUCIDATE THE PROTEOMIC INFLUENCES OF CONTRAST IN MR CANCER IMAGING IN AN EFFORT TO IMPROVE CLINICAL BREAST CANCER CARE BY ENABLING CLINICIANS TO QUICKLY ESTABLISH OR MODIFY TREATMENT REGIMENS BASED ON NON-INVASIVE ASSESSMENT OF CELLULAR-LEVEL TUMOR STATUS.

## BODY

THE TRAINING PLAN PROPOSED IN THE APPROVED STATEMENT OF WORK INCLUDES COURSEWORK, LABORATORY TRAINING, AND CULTIVATION OF A BROAD-BASED KNOWLEDGE OF CANCER, BREAST CANCER AND IMAGING SCIENCE ISSUES VIA ATTENDANCE OF RELEVANT SEMINARS AND CONFERENCES. DURING THE FIRST AWARD YEAR, THE SUPPORT PROVIDED BY THIS TRAINING GRANT WAS FOR THE PI TO EARN A M.S. DEGREE FROM THE VANDERBILT UNIVERSITY (VU) GRADUATE SCHOOL, COMPLETE LABORATORY TRAINING AS OUTLINED IN THE STATEMENT OF WORK, AND TO REFINE AND VALIDATE MR ACQUISITION AND ANALYSIS METHODS IN AN ANIMAL MODEL OF BRAIN CANCER. DURING THE SECOND AWARD YEAR, THE PI CONTINUED TO WORK CLOSELY WITH VANDERBILT UNIVERSITY INSTITUTE OF IMAGING AND BIOMEDICAL RESEARCH (VUIIR) FACULTY MENTORS TO DEVELOP COMPUTATIONAL TECHNIQUES FOR THREE-DIMENSIONAL RECONSTRUCTION AND CO-REGISTRATION OF MR AND MALDI DATA VIA PROCESSES DESCRIBED IN THE STATEMENT OF WORK. IN ADDITION, THE PI BEGAN COLLABORATION WITH THE VU TUMOR MICROENVIRONMENT NETWORK, AN INTERDISCIPLINARY NETWORK OF SCIENTISTS FUNDED BY THE NATIONAL CANCER INSTITUTE TO ELUCIDATE MECHANISMS OF TUMOR-HOST INTERACTIONS IN CANCER, TO FURTHER REFINE METHODS FOR THE FUSION OF MULTI-MODALITY IMAGING DATASETS.

THE RESEARCH PLAN PROPOSED IN THE APPROVED STATEMENT OF WORK DURING THE SECOND AWARD YEAR INCLUDES DEVELOPMENT OF PROTOCOLS FOR MR IMAGING OF BREAST CANCER METASTASIS TO BONE IN AN INTRA-TIBIAL MOUSE MODEL OF METASTATIC BREAST CANCER. WORKING WITH THE VU TUMOR MICROENVIRONMENT NETWORK FOR SMALL ANIMAL IMAGING, MR PROTOCOLS WERE OPTIMIZED AND MULTI-PARAMETRIC MR DATA WAS COLLECTED FOR EIGHT MICE, EACH WITH A SINGLE HIND LIMB TUMOR (SUCH THAT IMAGING OF THE CONTRALATERAL HIND LIMB MAY SERVE AS CONTROL DATA), AT EACH OF THREE DIFFERENT TIME POINTS. THE MICE WERE THEN PREPARED FOR COLLECTION OF PROTEOMIC DATA, A PROCESS THAT IS CURRENTLY BEING DEVELOPED IN CONTRAST TO COREGISTRATION TECHNIQUES EMPLOYED DURING THE FIRST AWARD YEAR USING MR IMAGING OF BRAIN CANCER AND FEATURING THE SKULL AS A NON-DEFORMABLE STEREOTACTIC FRAME. COMPARISON OF PROTEOMIC AND MR HIND LIMB DATA IN THIS BREAST CANCER MODEL WILL INCORPORATE BOTH RIGID AND NON-RIGID METHODS DEVELOPED DURING THE SECOND AWARD YEAR IN ORDER TO FUSE THE TWO DATASETS. REGISTRATION ON FIDUCIAL MARKERS IN THE ABSENCE OF A NON-DEFORMABLE STEREOTACTIC FRAME (FIGURE 1, APPENDIX). ANALYTICAL METHODS DEVELOPED DURING THE FIRST AWARD YEAR, DESCRIBED IN THE STATEMENT OF WORK, WILL BE APPLIED TO THE RESULTING HYBRID MULTI-MODAL DATA SETS. DATA ANALYSIS WILL INCLUDE IDENTIFICATION OF SPECIFIC (GROUPS OF) PROTEINS THAT MOST STRONGLY CORRELATE WITH VU TUMOR MICROENVIRONMENT NETWORK PARAMETRIC MRI DATA.

## KEY RESEARCH ACCOMPLISHMENTS

KEY RESEARCH ACCOMPLISHMENTS EMANATING FROM THIS TRAINING GRANT DURING THE FIRST YEAR WILL INCLUDE THE FOLLOWING:

- COMPLETION OF GRADUATE-LEVEL DIDACTIC COURSEWORK TO SUPPORT BREAST CANCER RESEARCH (E.G., CANCER IMAGING, QUANTITATIVE MAGNETIC RESONANCE IMAGING, AND MALDI-TOF IMAGE REGISTRATION CLASSES)
- COMPLETION OF LABORATORY TRAINING ON A 9.4 T VARIAN INOVA MR SCANNER
- COMPLETION OF LABORATORY TRAINING ON A LEICA CM3600 CRYOMACROTOME
- COMPLETION OF LABORATORY TRAINING ON AN AUTOFLEX III BRUKER DALTONICS LINEAR ION-TOF-FLIGHT MASS SPECTROMETER
- ADDITIONAL SUPPORTIVE TRAINING VIA ATTENDANCE OF REGULAR SEMINARS SPONSORED BY THE VANDERBILT UNIVERSITY INSTITUTE OF IMAGING SCIENCE, THE VANDERBILT UNIVERSITY CENTER FOR CELLULAR AND MOLECULAR IMAGING, THE VANDERBILT UNIVERSITY CENTER FOR TRANSLATIONAL RESEARCH, AND MULTIPLE ACADEMIC DEPARTMENTS
- REFINEMENT AND VALIDATION OF THREE-DIMENSIONAL RECONSTRUCTION AND INTER-SLICE CORRELATION OF SPECIMEN VOLUMES FROM MALDI DATA ON MULTIPLE TWO-DIMENSIONAL SPECIMEN SLICES
- REFINEMENT AND VALIDATION OF METHODS FOR CO-REGISTRATION OF MR AND MALDI DATA
- APPLICATION OF PRINCIPAL COMPONENT ANALYSIS TO HYBRID MALDI/MRI DATA SETS TO IDENTIFY COMMON MULTI-SPECTRAL BASIS SETS
- APPLICATION OF LINEAR REGRESSIVE AND CORRELATION ANALYSIS TECHNIQUES TO DETERMINE THE RELATIONSHIP BETWEEN PROTEOMIC AND DIFFUSION METRICS

KEY RESEARCH ACCOMPLISHMENTS EMANATING FROM THIS TRAINING GRANT DURING THE SECOND YEAR WILL INCLUDE THE FOLLOWING:

- COMPLETION OF LABORATORY TRAINING ON A 7 T 16-CM BORE VARIAN SCANNER
- COMPLETION OF TRAINING IN THE CARE OF MICE USED IN SERIAL IMAGING STUDIES
- OPTIMIZATION OF PROTOCOLS FOR GADOLINIUM CONTRAST-ENHANCED AND T1-, T2-, AND T2\*-WEIGHTED MR IMAGING OF HIND LIMB TUMORS IN A MOUSE MODEL OF METASTATIC BREAST CANCER
- ACQUISITION OF 24 MULTI-PARAMETRIC MOUSE HIND LIMB TUMOR MR DATASETS
- DEVELOPMENT OF METHODS FOR RIGID AND NON-RIGID CO-REGISTRATION OF IN VIVO MRI, MALDI VOLUME DATA IN A MOUSE MODEL OF METASTATIC BREAST CANCER
- ADDITIONAL SUPPORTIVE TRAINING VIA ATTENDANCE OF REGULAR SEMINARS SPONSORED BY THE VANDERBILT UNIVERSITY INSTITUTE OF IMAGING SCIENCE, THE VANDERBILT UNIVERSITY CENTER FOR CELLULAR AND MOLECULAR IMAGING, THE VANDERBILT UNIVERSITY CENTER FOR TRANSLATIONAL RESEARCH, AND MULTIPLE ACADEMIC DEPARTMENTS

- ONGOING PREPARATION FOR DOCTORAL DISSERTATION PROPOSAL AND DEFENSE

## REPORTABLE OUTCOMES

THE REPORTABLE OUTCOMES THAT HAVE RESULTED FROM THIS TRAINING GRANT IN THE FIRST YEAR INCLUDE THE FOLLOWING:

- ATTAINMENT OF THE DEGREE OF MASTER OF SCIENCE IN THE FIELD OF BIOMEDICAL ENGINEERING FROM THE VANDERBILT UNIVERSITY GRADUATE SCHOOL
- PRESENTATION OF RESEARCH METHODS AND RESULTS TO THE VUIIS FACULTY, STAFF, AND STUDENTS AT THE 2009 ANNUAL VUIIS RESEARCH RETREAT [4]

THE REPORTABLE OUTCOMES THAT HAVE RESULTED FROM THIS TRAINING GRANT IN THE SECOND YEAR INCLUDE THE FOLLOWING:

- PRESENTATION OF RESEARCH METHODS AND RESULTS TO THE VUIIS FACULTY, STAFF, AND STUDENTS AT THE 2010 ANNUAL VUIIS RESEARCH RETREAT [5]
- CO-INVESTIGATION OF ORIGINAL RESEARCH PRESENTED BY ERIN SEELEY, PH.D., VANDERBILT UNIVERSITY SPECTROMETRY RESEARCH CENTER, TO THE 58TH ASMS CONFERENCE ON MASS SPECTROMETRY AND ALLIED TOPICS [6]

## CONCLUSION

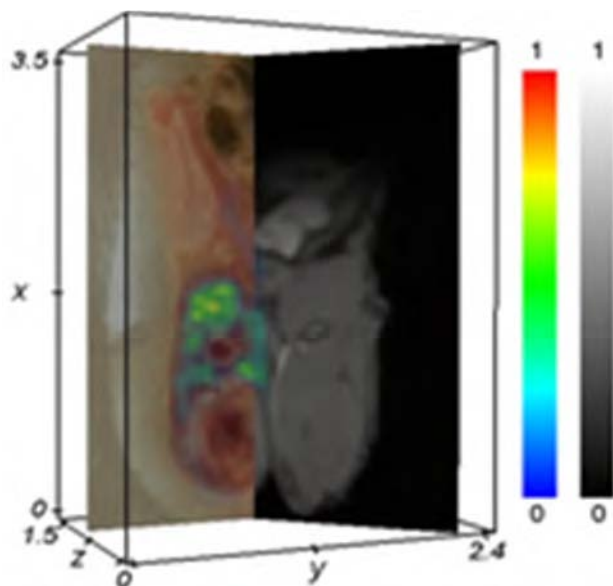
IN THE FIRST YEAR OF THIS STUDY, MALDI AND DIFFUSION-WEIGHTED (DW) MRI DATA WERE EXAMINED IN A C6 RAT BRAIN TUMOR MODEL (FIGURE 2 OF APPENDIX). SEVERAL HUNDRED REGIONS OF INTEREST WERE IDENTIFIED IN COREGISTERED MALDI/MR DATASETS OBTAINED FROM MULTIPLE ANIMALS WERE FOUND TO HAVE SIGNIFICANTLY DIFFERENT STATISTICALLY SIGNIFICANT LINEAR CORRELATIONS BETWEEN PROTEIN SIGNATURE INTENSITY AND DIFFUSION COEFFICIENT (FIGURE 3 OF APPENDIX) IN BOTH HEALTHY AND TUMOROUS TISSUES. THESE RESULTS INDICATE THAT PROTEIN CONTENT MAY SIGNIFICANTLY AFFECT CONTRAST IN DIFFUSION-WEIGHTED MR IMAGING. IN THE SECOND AWARD YEAR, MORE COMPLEX COREGISTRATION TECHNIQUES WERE DEVELOPED TO ACHIEVE FUSION OF MR AND PROTEIN DATA IN A MOUSE MODEL OF BREAST CANCER METASTASIS. IN THIS ANIMAL MODEL, COREGISTRATION OF DATA FROM THE TWO DIFFERENT IMAGING MODALITIES WAS ACHIEVED USING THE USE OF THE TIBIA AND OTHER FIDUCIAL MARKERS WITHIN DEFORMABLE HIND LIMB DATA, PROVIDING THE COMPUTATIONAL BENEFIT OF A NON-DEFORMABLE STEREOTACTIC FRAME SUCH AS THE SKULL. THIS ANALYSIS MORE CLOSELY MIMICS THE COMPUTATIONAL CHALLENGES OF APPLICATION OF THESE TECHNIQUES TO HUMAN BREAST CANCER DATA, WITH RESPECT TO THE CONFOUNDING NATURE OF COREGISTRATION OF DEFORMABLE HUMAN BREAST TISSUE. SERIAL MULTI-PARAMETRIC MR DATA WERE OBTAINED FROM MICE WITH HIND LIMB TUMORS ORIGINATING FROM BREAST CANCER CELLS. ACQUISITION OF PROTEIN DATA FROM THESE MICE IS UNDERWAY. UPON COMPLETION OF COLLECTION OF PROTEIN DATA, THE MR AND PROTEIN DATA WILL BE COREGISTERED AND SUBJECTED TO ANALYTICAL TECHNIQUES DEVELOPED DURING THE FIRST YEAR. DATA ANALYSIS WILL FOCUS ON IDENTIFICATION OF SPECIFIC (GROUPS OF) PROTEINS THAT CORRELATE WITH VARIATIONS IN MULTI-PARAMETRIC MRI DATA. THIS WORK REPRESENTS A BASIC STEP TOWARDS THE LONG-TERM OBJECTIVE OF FACILITATING CLINICAL ASSESSMENT OF TUMOR SIZE USING NON-INVASIVE IMAGING TECHNIQUES.

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## APPENDIX: SUPPLEMENTARY FIGURES

THE FOLLOWING FIGURES ILLUSTRATE REPRESENTATIVE DATA AND RESULTS AS DESCRIBED IN THE



**Figure 1:** COREGISTERED OPTICAL IMAGE OF MOUSE TISSUE (RBG), MRI (GRAYSCALE), AND M. (FALSE COLOR) DATA FOR PROTEIN OF MOLECULAR MASS 11838 DA. LEFT COLORBAR: REL. CONCENTRATION. RIGHT COLOR-BAR: RELATIVE MR SIGNAL INTENSITY.



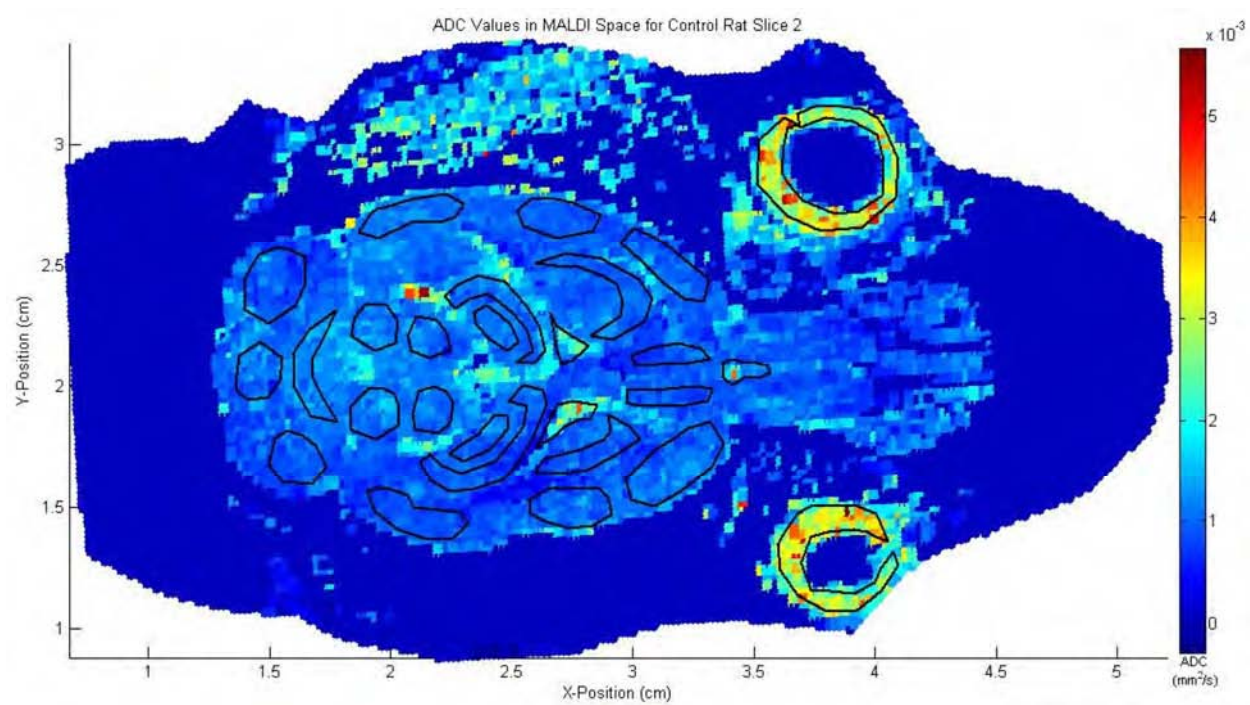


FIGURE 2: REPRESENTATIVE REGIONS OF INTEREST IN ADC DATA CO-REGISTERED TO MALDI SPACE. REGIONS OF INTEREST WERE MANUALLY DELINEATED TO DEFINE SELECTED ANATOMICAL STRUCTURES IN [7].

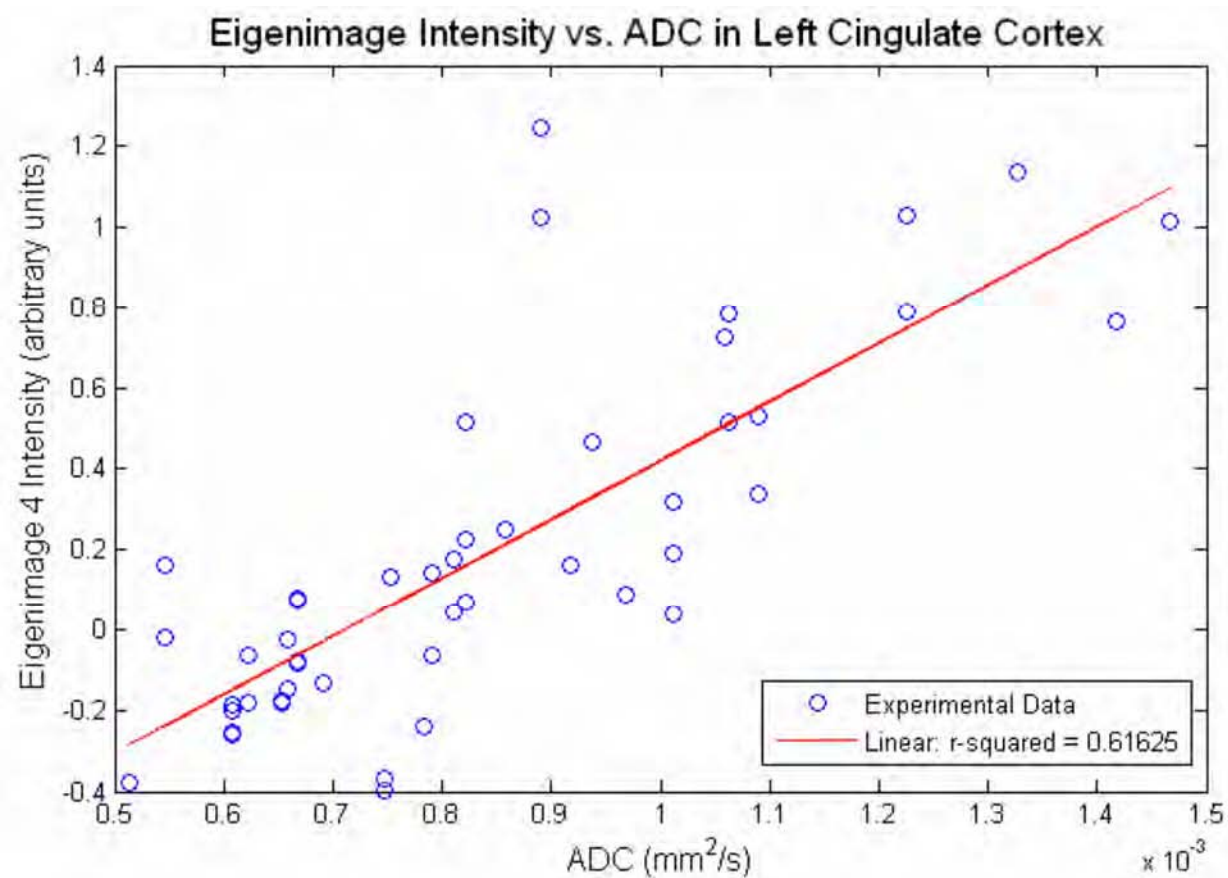


FIGURE 3: SCATTER PLOT CHARACTERIZING THE RELATIONSHIP BETWEEN THE ADC AND EIGENIMAGE 4 INTENSITY IN A REGION OF INTEREST DELINEATING THE LEFT CINGULATE CORTEX. THE DATA SHARE AN APPROPRIATE LINEAR RELATIONSHIP WITH A PEARSON CORRELATION COEFFICIENT OF 0.7850 AT A CONFIDENCE LEVEL OF 0.05 (TO FIVE SIGNIFICANT DIGITS).